77 of SEQ ID NO: 37 with Ala, Pro substituted at position 108 of SEQ ID NO: 37 with Gly, or (Asp substituted at position 28 of SEQ ID NO: 37 with Thr, Lys substituted at position 32 of SEQ ID NO: 37 with Gln, Glu substituted at position 45 of SEQ ID NO: 37 with Ser, Pro substituted at position 108 of SEQ ID NO: 37 with Gly). --

REMARKS

Claims 1-28, 32-34 and 47 are pending in the above-captioned application. With this response, claims 1 and 15 are canceled and replaced with new claims 48 and 50. New claim 49 is added. Therefore after entry of this response, pending in the application are claims 2-14, 16-28, 32-34 and 47-50. No new matter has been introduced with these changes. Full support for the amendments is found throughout the specification and is discussed in more detail below.

The title had been amended as requested by the Examiner.

The specification has been amended to include the priority claim as requested by the Examiner.

The specification within the paragraph on page 35, line 26 to page 36, line 1 has been corrected as requested by the Examiner. Support for this change is found on page 35, line 21.

Applicants submit herewith a Substitute Declaration as requested by the Examiner to comply with 37 C.F.R. §1.67.

The Abstract has been amended as requested by the Examiner. Support for this amendment is found on page 18, line 36 to page 19, line 1.

Applicants acknowledge the objection to the drawings; drawings in compliance with the Rules will be submitted in a timely fashion.

A copy of the IDS filed July 9, 1999 and the date stamped postcard confirming receipt of the IDS in the USPTO is attached hereto.

Rejections under Section 112, first paragraph

Claims 1-15, 32-34 and 47 are rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. The Examiner states that the specification does not reasonably provide enablement

for any recombinant birch pollen antigen. Applicants respectfully traverse the rejection.

The specification contains an enabling description of how to make and use the full breadth of the claimed invention. The specification describes in detail the essential criteria for obtaining the mutant allergens of the claimed invention (see e.g., pages 19-20), and clearly describes throughout the specification the methods and techniques that may be used by the skilled practitioner to obtain the mutant allergens. In addition, detailed examples of how to make and use the Bet v1 and Ves v5 mutants are provided. The application has provided a description that fully enables a practitioner of ordinary skill to obtain a mutant of the invention having the desired characteristics, i.e., the three-dimensional structure of the mutant allergen is essentially the same as that of the naturally occurring allergens from which it was derived, however the mutant allergen has a reduced IgE-binding ability compared to that of the naturally occurring allergen. The principles that guide the methods used to obtain the mutant allergens of the invention are applicable to any known allergen, regardless of its source.

In addition, the examples provide detailed guidance for obtaining mutant allergens from two very different sources of allergen, i.e., inhalation allergens and venom allergens, and specifically, birch pollen antigen Bet v 1 and vespid venom Ves v 5, respectively. Thus applicants have shown that the principles taught in the specification may be applied to a wide range of allergen sources. Accordingly, the invention as claimed and described may be applied to any allergen source.

A practitioner would be able to readily apply the techniques taught in the specification, without undue experimentation, to produce mutant recombinant allergens from any allergen known in the art. The proscription against undue experimentation is not a proscription against some experimentation. The test of enablement is not whether any experimentation is necessary but whether, if experimentation is necessary, it is undue. *In re Angstadt* 190 USPQ 214, 219 (CCPA 1976); MPEP 2164.01. Although some experimentation may be required to produce each allergen within the scope of the claims, such experimentation, although complex, is not necessarily "undue". As discussed above, the specification describes in detail the essential criteria for obtaining the mutant allergens of the claimed invention. The methods and techniques used to practice the invention, including analyzing sequence homologies, studying protein structures, identifying suitable patches of conserved amino acid residues, and determining IgE binding levels,

are understood by those of skill in the art, given the level of complexity inherent in the art, to require some experimentation. However, given the level of knowledge of those skilled in the art, this does not constitute undue experimentation. Further, applicants need not teach "all possible" amino acid substitutions within a given sequence that will meet the requirements of the invention. Applicants need only describe the invention in terms such that one skilled in the art can use the claim invention. This applicants have done.

The specification discloses that substitutions of certain amino acids in the common patch regions maintain the structural conformation and reduce the IgE binding activity of the mutant allergen compared to those properties in the naturally occurring allergen. The specification further describes how to obtain mutants of diverse types of allergens. This is sufficient to meet the requirements of Section 112. The specification enables the full scope of the claimed invention and meets the requirements of Section 112.

Claims 33-34 and 47 are rejected under 35 U.S.C. § 112, first paragraph because the specification allegedly does not provide guidance in formulating the claimed pharmaceutical compositions. The Examiner takes the position that the specification is not enabling because it contains no *in vivo* data with respect to efficacy, dosage and stability. Applicants respectfully traverse the rejection.

The specification fully meets the requirements of Section 112 with regard to formulating the claimed pharmaceutical compositions. According to U.S. patent law, it is not necessary to provide *in vivo* data in order to establish that a composition will have an *in vivo* effect; *in vitro* data is generally sufficient for this purpose. *Cross v. Iizuka,* 224 USPQ 739, 747 (Fed. Cir. 1985). All that is required to meet the requirements of Section 112 is that there be a correlation between the in vitro and in vivo models described in the specification and the asserted use. The specification meets this requirement. The specification contains several detailed examples of *in vitro* data which clearly suggest that the claimed allergens would have a therapeutic effect *in vivo*. For example, on page 31, applicants describe an in vitro assay which examines the Ig E binding properties of an allergen mutant. The *in vitro* data were obtained using *in vivo* effector molecules from a therapeutic target group (IgE serum pools). It is well known in the art that IgE binds *in vivo* to allergens thereby inducing a cascade of reaction leading to an allergic response. Allergy

vaccination is also well known in the art as the concept of administering controlled dosages of allergens obtained from the natural source and that such administration may lead to side effects due to the cascade reaction. Thus there is a well known demonstrated correlation between the in vitro demonstration of binding activity and the asserted use of a mutant allergen in a pharmaceutical composition.

Furthermore, it would be well within the capabilities of a practitioner of ordinary skill in the art to determine an appropriate dosage and appropriate storage/production conditions for the claimed allergens in order to ensure long-term stability of the claimed allergens without an undue amount of experimentation.

In view of the foregoing remarks, applicants submit that the Section 112 rejections of claims 1-15, 32-34 and 47 cannot be maintained and respectfully request that the rejections be withdrawn.

Rejections under Section 112, second paragraph

Claims 1-2, 32-34 and 47 have been amended to more clearly define the claimed invention with respect to the specific language rejected by the Examiner, except as discussed below. Support for the language of claim 1, now rewritten as new claim 48, can be found on page 17, lines 13-17, page 20, lines 6-14 and page 14, line 35 to page 15, line 3. Support for the language of claim 15, now rewritten as new claim 50, can be found on page 16, lines 17-22.

With respect to the language in claim 2 "more than 70% identity", applicants respectfully request clarification as to the rejection for alleged lack of antecedent basis. The claim recites that the amino acid residues "are conserved with more than 70% identity". This language refers back to and further defines the term "conserved" in claim 48 (former claim 1). Support for this language is also found on page 14, lines 22-26 of the specification.

With respect to claim 1, now rewritten as new claim 48, the language "having essentially the same" is fully defined in the specification and will be understood by those of skill in the art to mean that the backbone structure is conserved between the mutant and the naturally occurring allergen (see page 17, lines 29-35).

In view of the amendments to the claims and the foregoing remarks, Applicants

respectfully request that the Section 112, second paragraph rejections be withdrawn.

Rejection under Section 102 "Gajhede *et al.*"

Claims 1-14 have been rejected as anticipated under 35 U.S.C. §102 (b) by Gajhede *et al.* The Examiner takes the position that the reference discloses the claimed non-naturally occurring mutant allergen. Applicants respectfully traverse the rejection.

Gajhede *et al.* disclose only a wild-type, unmutated allergen (Bet v1). Gajhede *et al.* does not disclose Bet v1 comprising a mutation, non-natural or otherwise, and certainly not the mutant allergen as described in claim 1. Since the allergens disclosed by Gajhede *et al.* are not identical to the claimed allergens, the claims are not anticipated by the reference.

Applicants respectfully request that the Section 102 rejection under Gajhede *et al.* be withdrawn.

Rejection under Section 103 Gajhede *et al.* Lowenstein *et al.* and Breiteneder *et al.*

Claims 1-14, 32-33 and 47 have been rejected as obvious 35 U.S.C. §103 (a) over Gajhede *et al.*, in view of Lowenstein *et al.* and Breiteneder *et al.* The Examiner alleges that it would have been obvious to select a residue on the surface of an allergen (Bet v1) in view of the allergen's three-dimensional crystal structure (as allegedly taught by Gajhede *et al.*) and introduce mutations which may reduce antibody (IgE) binding of the antigen (as allegedly taught by Lowenstein *et al.*). The Examiner also takes the position that it would have been obvious to use the mutated allergens in a pharmaceutical composition as allegedly disclosed by Breiteneder *et al.* Applicants respectfully traverse the rejection.

The references in combination do not teach or suggest the claimed invention because none of the primary reference or secondary references suggest introducing mutations into allergens.

Gajhede et al. discloses the three-dimensional structure and the specific structural elements of the protein molecule of the major birch allergen Bet v 1. The possibility of certain areas being B-cell epitopes is discussed, and that these epitopes may also be responsible for at least some of the mechanisms involved in cross-reactivity seen in birch allergics upon exposure to related allergens from other trees of the Fagales order. Based on experimental suggestions that an antibody

will cover an estimated area of 600-900 Å^2 and the comparison of amino acid sequence similarities within the tree order *Fagales*, three areas on the Bet v1 molecular surface are suggested to harbor such cross-binding epitopes. However, the reference is only concerned with naturally occurring isoforms and the relationship between cross-reactive epitopes of related species.

Nothing in Gajhede et al. teaches or anticipates non-naturally occurring mutant allergens. Gajhede et al. neither teaches or makes obvious which amino acids to select for substitution, nor the amino acids to be substituted, nor that such allergen mutants provide specific beneficial useful properties.

Løwenstein et al. investigates the variation in naturally occurring allergen isoforms and their different IgE-binding properties. In particular, Lowenstein describes the heterogeneity of Bet v1 isoallergens, which results in differences in antibody and T cell recognition and cross-reactivity. Lowenstein suggests that different antigenicity of isoallergens and structurally similar allergens results from amino acid substitutions that induce local perturbations on molecular surfaces, leading to different epitope structures (see Lowenstein, p. 287). Specifically, Lowenstain states that "however, as more amino acid substitutions are induced, the recombinant isoallergens show differences in their antibody-binding properties" (page 287, column 1, 2nd paragraph). Thus, Lowenstein indicates that even though amino acid substitutions alter the surface and this may have an impact on binding properties of antibodies, the correlation between substitutions and effect on antibody binding is at best uncertain.

Lowenstein's prediction that "the diversity of epitope structures of isoallergens might have important implications for the use of peptides and recombinant proteins in immunotherapy," or the speculation that amino acid substitutions "may also have an effect" on the T-cell epitopes do not provide one of skill in the art with any expectation regarding what effect amino acid substitutions will have on structure and function of the allergens. Such statements express uncertainty about what the consequences of these changes might be and by no means amount to a suggestion of the claimed allergens. Furthermore, Lowenstein relates to isoallergens which are naturally occurring variants. Thus, there is no objective disclosure which would enable a practitioner of average skill in the art to create the claimed mutants, much less suggest that such mutants are effective for allergy therapy.

Breiteneder et al. discloses a DNA and polypeptide with Bet v 1- like allergenicity.

In particular, Breiteneder et al. teaches synthetic polypeptides having corresponding allergenicity to natural allergens (see column 3, lines 23-25, lines 37-39). The envisaged therapeutic use and benefit of pharmaceuticals comprising the disclosed polypeptide (natural isoforms) is that of reduced risk of sensitizing the patient to unwanted components, (see column 9, lines 7-9), and not reducing the side effects due to reduced IgE-binding. Breiteneder et al. does not disclose non-natural mutants of the claimed invention nor does Breiteneder et al. suggest the use of such mutants in therapeutic applications.

In summary, all three references are concerned with naturally occurring isoforms. Neither of the references alone or in combination is concerned with non-naturally occurring mutants None of the references is concerned with the preservation of the three dimensional structure upon substitution of an amino acid. They neither teach *nor* suggest allergen mutants according to the claimed invention. The cited documents alone or in combination do not provide a reasonable expectation of success for a skilled artisan to obtain mutants according to the claimed invention. Applicants submit that the obviousness rejection cannot stand and should be withdrawn.

Rejection under Section 102 "Breitender *et al.*"

Claims 1, 3, 10-13 and 32-33 stand rejected as anticipated by Breitender *et al.* Applicants respectfully traverse the rejection.

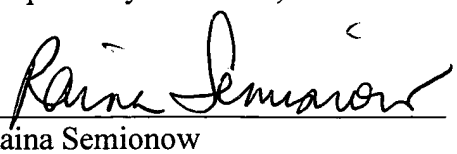
Applicants submit that the rejection is inappropriate. Other than a simple statement that the claims are rejected in view of the reference, the Examiner offers no explanation of the grounds for the rejection. However, the Rules (specifically 37 C.F.R. §§104(a) 2 and (c) 2) and MPEP § 707 require the Examiner to provide reasons for a rejection, particularly if the pertinence of the reference is not apparent.

Breitender *et al.* discloses constructs for expression of various allergens. Breitender *et al.* does not appear to describe allergen mutants, and certainly not the specific, claimed mutant variants of the invention. Therefore the pertinence of Breitender *et al.* is not apparent and applicants are unable to distinctly and specifically address the rejection. Applicants respectfully request clarification of the rejection.

CONCLUSION

In view of the foregoing amendments and remarks, applicants submit that the claims are in form for allowance. A notice to that effect is earnestly solicited. A Petition for Extension of Time for response for two months and the appropriate fee are enclosed.

Respectfully submitted,



Raina Semionow
Reg. No. 39,022
Attorney for Applicants

DARBY & DARBY, P.C.
805 Third Avenue
New York, N.Y. 10022
Phone (212) 527-7700

M:\4305\1E144\PXS0013.WPD

Customer No.: 07278

PATENT TRADEMARK OFFICE

Docket No: 4305/1E144-US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Hans Henrik Ipsen et al.

Serial No.: 09/270,910 Art Unit: 1644

Confirmation No.:

Filed: 3/16/99 Examiner: P. Huynh

For: NOVEL RECOMBINANT ALLERGENS

MARKED UP AMENDMENTS

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

July 13, 2001

Sir:

This is in connection with the response to the Official Action dated February 13, 2001. Please amend the above-identified application as follows:

In the title:

Please amend the title:

[NOVEL] RECOMBINANT ALLERGENS

In the specification:

Please amend page 35, line 26 to page 36, line 1 as follows:

Figure 7 shows the inhibition of the binding of biotinylated recombinant Bet v 1 (SEQ ID NO: 37) to serum IgE from a pool of allergic patients by non-biotinylated Bet v1 (SEQ ID NO: 37) and by Bet v1

Glu60Ser mutant (SEQ ID NO: 37). In contrast to the Glu45Ser, Pro108Gly and Asn28Thr+Lys32Gln mutants (SEQ ID NO: 37), the substitution glutamic acid 60 to serine, does not [shown] show any significant effect on the IgE- binding properties of Bet v 1 Glu60Ser mutant (SEQ ID NO: 37). This indicates that substitutions outside the defined *Fagales* common patches only have a marginal effect on the binding of specific serum IgE supporting the concept that conserved allergen molecular surface areas harbor [harbours] dominant IgE-binding epitopes.

In the Abstract:

Please amend the abstract as follows:

[NOVEL RECOMBINANT ALLERGENS]

Novel recombinant allergens are disclosed. The allergens are non-naturally occurring mutants derived from naturally-occurring allergens. The overall α-carbon backbone tertiary structure of the allergens is essentially preserved. Also disclosed are [method] methods for preparing [such] the recombinant allergens as well as [uses thereof] the use of the recombinant allergens for the treatment of allergic reactions .

In the claims:

2. (Twice Amended) A recombinant [Recombinant] allergen according to claim 48 [1], [characterised in that it is] obtainable by

a) identifying amino acid residues in a naturally occurring allergen which are conserved with more than 70% identity in all known of the homologous proteins within the taxonomic order from which said naturally occurring allergen originates;

b) defining at least one patch of conserved amino acid residues being coherently connected over at least 400 $Å^2$ of the surface of the three-dimensional structure of the naturally occurring allergen molecule as defined by having a solvent accessibility of at least 20%, said at least one patch comprising at least one B cell epitope; and

c) substituting at least one amino acid residue in said at least one patch with [by] another amino acid which is not conserved [being non-conservative in the particular position],wherein [while essentially preserving] the [overall] α-carbon backbone tertiary structure of the allergen molecule is essentially preserved.

3. (Twice Amended) A recombinant [Recombinant] allergen according to claim 48 [1], wherein [characterised in that] the specific IgE binding to the mutant [mutated] allergen is reduced by at least 5%, preferably at least 10%.

4. (Twice Amended) A recombinant [Recombinant] allergen according to [any of] claim 48 [1], wherein [characterised in that when comparing the α-carbon backbone tertiary structures of the mutant and the naturally occurring allergen molecules,] the average root mean square deviation of the atomic coordinates comparing the α-carbon backbone tertiary structures of the mutant and the naturally occurring allergen molecules is below 2Å.

5. (Twice Amended) A recombinant [Recombinant] allergen according to claim 2, wherein [characterised in that] said at least one patch comprises atoms of 15-25 amino acid residues.

6. (Twice Amended) A recombinant [Recombinant] allergen according to [any one of] claim 2, wherein [characterised in that] the amino acid residues of said at least one patch are ranked with respect to solvent accessibility, and one or more amino acids among the more solvent accessible ones are substituted.

7. (Amended) A recombinant [Recombinant] allergen according to claim 6, wherein [characterised in that] one or more amino acid residues of said at least one patch having a solvent accessibility of 20-80% are substituted.

8. (Twice Amended) A recombinant [Recombinant] allergen according to [any one of] claim 2, wherein [characterised in that] 1-5 amino acid residues per 400$Å^2$ in said at least one patch are substituted.

9. (Twice Amended) A recombinant [Recombinant] allergen according to [any one of] claim 2, wherein [characterised in that] the substitution of one or more amino acid residues in said B cell epitope or said at least one patch is carried out by site-directed mutagenesis.

10. (Twice Amended) A recombinant [Recombinant] allergen according [any one of] claim 48 [1], [characterised in that it] wherein the allergen is derived from an inhalation allergen.

11. (Amended) A recombinant [Recombinant] allergen according to claim 10, [characterised in that it] wherein the allergen is derived from a pollen allergen.

12. (Amended) A recombinant [Recombinant] allergen according to claim 10, [characterised in that it] wherein the allergen is derived from a pollen allergen originating from the taxonomic order of *Fagales*, *Oleales* or *Pinales*.

13. (Amended) A recombinant [Recombinant] allergen according to claim 12, [characterised in that it] wherein the allergen is derived from *Bet v 1*.

14. (Amended) A recombinant [Recombinant] allergen according to claim 13, wherein [characterised in that] at least one amino acid residue of said B cell epitope or said at least one patch is substituted.

16. (Amended) A recombinant [Recombinant] allergen according to claim 11, [characterised in that it] wherein the allergen is derived from a pollen allergen originating from the taxonomic order of *Poales*.

17. (Amended) A recombinant [Recombinant] allergen according to claim 11, [characterised in that it] wherein the allergen is derived from a pollen allergen originating from the taxonomic order of *Asterales* or *Urticales*.

18. (Amended) A recombinant [Recombinant] allergen according to claim 10, [characterised in that] wherein the allergen is derived from a house dust mite allergen.

19. (Amended) A recombinant [Recombinant] allergen according to claim 18, [characterised in that] wherein the allergen is derived from a mite allergen originating from *Dermatophagoides*.

20. (Amended) A recombinant [Recombinant] allergen according to claim 10, [characterised in that] wherein the allergen is derived from a cockroach allergen.

21. (Amended) A recombinant [Recombinant] allergen according to claim 10, [characterised in that] wherein the allergen is derived from an animal allergen.

22. (Amended) A recombinant [Recombinant] allergen according to claim 21, [characterised in that] wherein the allergen is derived from an animal allergen originating from a cat, dog or horse.

23. (Twice Amended) A recombinant [Recombinant] allergen according to [any one of] claim 48 [1], [characterised in that] wherein the allergen is derived from a venom allergen.

24. (Amended) A recombinant [Recombinant] allergen according to claim 23 [characterised in that] wherein the allergen is derived from a venom allergen originating from the taxonomic order of *Hymenoptera*.

25. (Amended) A recombinant [Recombinant] allergen according to claim 24 [characterised in that] wherein the allergen is derived from a venom allergen from the taxonomic order of Vespidae, Apidae and Formicoidae.

26. (Twice Amended) A recombinant [Recombinant] allergen according to [any one of] claim 23, [characterised in that] wherein the allergen is derived from *Ves v* 5.

27. (Twice Amended) A recombinant [Recombinant] allergen according to claim 23, [characterised in that] wherein at least one amino acid is substituted.

28. (Twice Amended) A recombinant [Recombinant] allergen according to [any one of] claim 25, [characterised in that] wherein the substitution is Lys72A1a or Tyr96A1a.

32. (Twice Amended) A recombinant [Recombinant] allergen according to [any of] claim 48 [1] for use as a pharmaceutical.

33. (Twice Amended) A pharmaceutical [Pharmaceutical] composition, comprising [characterised in that it comprises] a recombinant allergen according to [any one of] claim 48 [1], optionally in combination with a pharmaceutically acceptable carrier and/or excipient, and optionally an adjuvant.

34. (Amended) A pharmaceutical composition according to claim 33, [characterised in that it is] in the form of a vaccine against allergic reactions elicited by a naturally occurring allergen in patients suffering from allergy.

47. (Amended) A pharmaceutical [Pharmaceutical] composition obtainable by the process according to claim 33 [36].

Respectfully submitted,

Raina Semionow
Reg. No. 39,022
Attorney for Applicants

DARBY & DARBY, P.C.
805 Third Avenue
New York, N.Y. 10022
Phone (212) 527-7700

M:\4305\1E144\RXS0852.WPD